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LEANNE MYNOTT
MANAGER EXAMINATION SUPPORT
AND SALES

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AUSTRALIA

Patents Act 1990

PROVISIONAL SPECIFICATION

Invention Title: **Modification of plant response to freezing and low temperature stress**

The invention is described in the following statement:

MODIFICATION OF PLANT RESPONSE TO FREEZING AND LOW TEMPERATURE STRESS

The present invention relates to nucleic acids or nucleic acid fragments encoding amino acid sequences for ice recrystalisation inhibition proteins in plants, and the use thereof for the modification of plant response to freezing and/or low temperature stress.

Plants have evolved a range of physiological and biochemical responses to freezing and low temperature stress. In plant species that are tolerant of freezing stress, exposure to lowering temperatures is accompanied by the accumulation of a characteristic set of proteins, called 'antifreeze proteins' (AFPs). Some AFPs act to depress the freezing point temperature allowing the plant to supercool. A class of AFPs, the ice recrystallisation inhibition proteins (IRIPs), confer freezing tolerance by inhibiting ice crystal growth, promoting the formation of small ice crystals in preference to large ice crystals that puncture membranes and disrupt the structure of macromolecular complexes. IRIP activity has been identified in extracts from a limited number of plant species, and the nucleotide sequence of one such IRIP from *Lolium perenne* has been reported.

As nucleic acid sequence encoding an IRIP has been isolated from only one species of plant, there is a need for materials useful in modifying the tolerance of freezing and low temperature stress, in a wide range of plants, and for methods for their use.

It is an object of the present invention to overcome, or at least alleviate, one or more of the difficulties or deficiencies associated with the prior art.

In one aspect, the present invention provides substantially purified or isolated nucleic acids or nucleic acid fragments encoding IRIPs from a Deschampsia species, preferably Antarctic hair-grass, *Deschampsia Antarctica*, or functionally active fragments or variants thereof.

The present invention also provides substantially purified or isolated nucleic acids or nucleic acid fragments encoding amino acid sequences for a class of

proteins which are related to IRIP or functionally active fragments or variants thereof. Such proteins are referred to herein as IRIP-like.

The individual or simultaneous enhancement or otherwise manipulation of IRIP or like gene activities in plants may enhance or otherwise alter the freezing and/or low temperature tolerance of plants.

The modification of plant freezing and/or low temperature tolerance based on the individual or simultaneous enhancement or otherwise manipulation of IRIP or like gene activities in plants has significant consequences for a range of applications in plant production and plant protection. For example, it has applications in increasing the range and productivity of plants.

Methods for the modification of plant freezing and/or low temperature tolerance may facilitate the production of, for example, plants with enhanced tolerance of freezing and/or low temperature stress.

The nucleic acid or nucleic acid fragment may be of any suitable type and includes DNA (such as cDNA or genomic DNA) and RNA (such as mRNA) that is single- or double-stranded, optionally containing synthetic, non-natural or altered nucleotide bases, and combinations thereof.

In a preferred embodiment of this aspect of the invention, the substantially purified or isolated nucleic acid or nucleic acid fragment encoding an IRIP or IRIP-like protein includes a nucleotide sequence selected from the group consisting of (a) sequences shown in Figures 1, 3, 5, 6, 8, 9, 11, 13, 14 and 16 hereto; (b) complements of the sequences recited in (a); (c) sequences antisense to the sequences recited in (a) and (b); and (d) functionally active fragments and variants of the sequences recited in (a), (b) and (c).

The term "isolated" means that the material is removed from its original environment (eg. the natural environment if it is naturally occurring). For example, a naturally occurring nucleic acid or polypeptide present in a living plant is not isolated, but the same nucleic acid or polypeptide separated from some or all of

the coexisting materials in the natural system, is isolated. Such nucleic acids could be part of a vector and/or such nucleic acids could be part of a composition, and still be isolated in that such a vector or composition is not part of its natural environment.

Such nucleic acid fragments could be assembled to form a consensus contig. As used herein, the term “consensus contig” refers to a nucleotide sequence that is assembled from two or more constituent nucleotide sequences that share common or overlapping regions of sequence homology. For example, the nucleotide sequence of two or more nucleic acid fragments can be compared and aligned in order to identify common or overlapping sequences. Where common or overlapping sequences exist between two or more nucleic acid fragments, the sequences (and thus their corresponding nucleic acid fragments) can be assembled into a single contiguous nucleotide sequence.

The term "purified" means that the nucleic acid or polypeptide is substantially free of other nucleic acids or polypeptides.

By “functionally active” in respect of a nucleic acid it is meant that the fragment or variant (such as an analogue, derivative or mutant) is capable of modifying the tolerance of freezing and/or low temperature stress in a plant. Such variants include naturally occurring allelic variants and non-naturally occurring variants. Additions, deletions, substitutions and derivatizations of one or more of the nucleotides are contemplated so long as the modifications do not result in loss of functional activity of the fragment or variant. Preferably the functionally active fragment or variant has at least approximately 80% identity to the relevant part of the above mentioned sequence, more preferably at least approximately 90% identity, most preferably at least approximately 95% identity. Such functionally active variants and fragments include, for example, those having nucleic acid changes which result in conservative amino acid substitutions of one or more residues in the corresponding amino acid sequence. Preferably the fragment has a size of at least 30 nucleotides, more preferably at least 45 nucleotides, most preferably at least 60 nucleotides.

By "functionally active" in respect of a polypeptide is meant that the fragment or variant has one or more of the biological properties of an IRIP or IRIP-like protein. Additions, deletions, substitutions and derivatizations of one or more of the amino acids are contemplated so long as the modifications do not result in loss of functional activity of the fragment or variant. Preferably the functionally active fragment or variant has at least approximately 60% identity to the relevant part of the above mentioned sequence, more preferably at least approximately 80% identity, most preferably at least approximately 90% identity. Such functionally active variants and fragments include, for example, those having conservative amino acid substitutions of one or more residues in the corresponding amino acid sequence. Preferably the fragment has a size of at least 10 amino acids, more preferably at least 15 amino acids, most preferably at least 20 amino acids.

The term "construct" as used herein refers to an artificially assembled or isolated nucleic acid molecule which includes the gene of interest. In general a construct may include the gene or genes of interest, a marker gene which in some cases can also be the gene of interest and appropriate regulatory sequences. It should be appreciated that the inclusion of regulatory sequences in a construct is optional, for example, such sequences may not be required in situations where the regulatory sequences of a host cell are to be used. The term construct includes vectors but should not be seen as being limited thereto.

The term "vector" as used herein encompasses both cloning and expression vectors. Vectors are often recombinant molecules containing nucleic acid molecules from several sources.

By "operatively linked" is meant that said regulatory element is capable of causing expression of said nucleic acid or nucleic acid fragment in a plant cell and said terminator is capable of terminating expression of said nucleic acid or nucleic acid fragment in a plant cell. Preferably, said regulatory element is upstream of said nucleic acid or nucleic acid fragment and said terminator is downstream of said nucleic acid or nucleic acid fragment.

By "an effective amount" it is meant an amount sufficient to result in an identifiable phenotypic trait in said plant, or a plant, plant seed or other plant part derived therefrom. Such amounts can be readily determined by an appropriately skilled person, taking into account the type of plant, the route of administration and other relevant factors. Such a person will readily be able to determine a suitable amount and method of administration. See, for example, Maniatis et al, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, the entire disclosure of which is incorporated herein by reference.

Genes encoding other IRIP or IRIP-like proteins for modifying the tolerance of plants to freezing and/or low temperature stress, either as cDNAs or genomic DNAs, may be isolated directly by using all or a portion of the nucleic acids or nucleic acid fragments of the present invention as hybridisation probes to screen libraries from the desired plant employing the methodology well known to those skilled in the art. Specific oligonucleotide probes based upon the nucleic acid sequences of the present invention may be designed and synthesized by methods known in the art. Moreover, the entire sequences may be used directly to synthesize DNA probes by methods known to the skilled artisan, such as random primer DNA labelling, nick translation, or end-labelling techniques, or RNA probes using available *in vitro* transcription systems. In addition, specific primers may be designed and used to amplify a part or all of the sequences of the present invention. The resulting amplification products may be labelled directly during amplification reactions or labelled after amplification reactions, and used as probes to isolate full length cDNA or genomic fragments under conditions of appropriate stringency.

In addition, short segments of the nucleic acids or nucleic acid fragments of the present invention may be used in protocols to amplify longer nucleic acid fragments encoding homologous genes from DNA or RNA. For example, polymerase chain reaction may be performed on a library of cloned nucleic acid fragments wherein the sequence of one primer is derived from the nucleic acid sequences of the present invention, and the sequence of the other primer takes advantage of the presence of the polyadenylic acid tracts to the 3' end of the mRNA precursor encoding plant genes. Alternatively, the second primer sequence

may be based upon sequences derived from the cloning vector. For example, those skilled in the art can follow the RACE protocol [Frohman *et al.* (1988) *Proc. Natl. Acad Sci. USA* 85:8998, the entire disclosure of which is incorporated herein by reference] to generate cDNAs by using PCR to amplify copies of the region between a single point in the transcript and the 3' or 5' end. Using commercially available 3' RACE and 5' RACE systems (BRL), specific 3' or 5' cDNA fragments may be isolated [Ohara *et al.* (1989) *Proc. Natl. Acad Sci USA* 86:5673; Loh *et al.* (1989) *Science* 243:217, the entire disclosures of which are incorporated herein by reference]. Products generated by the 3' and 5' RACE procedures may be combined to generate full-length cDNAs.

In a second aspect of the present invention there is provided a substantially purified or isolated IRIP or IRIP-like polypeptide from a Deschampsia species, preferably from Antarctic hair-grass, *Deschampsia Antarctica*; and functionally active fragments and variants thereof.

In a preferred embodiment of this aspect of the invention, the substantially purified or isolated IRIP or IRIP-like polypeptide includes an amino acid sequence selected from the group consisting of sequences shown in Figures 2, 4, 7, 10, 12 and 15 hereto and functionally active fragments and variants thereof.

In a further embodiment of this aspect of the invention, there is provided a polypeptide recombinantly produced from a nucleic acid or nucleic acid fragment according to the present invention. Techniques for recombinantly producing polypeptides are well known to those skilled in the art.

Availability of the nucleotide sequences of the present invention and deduced amino acid sequences facilitates immunological screening of cDNA expression libraries. Synthetic peptides representing portions of the instant amino acid sequences may be synthesized. These peptides may be used to immunise animals to produce polyclonal or monoclonal antibodies with specificity for peptides and/or proteins comprising the amino acid sequences. These antibodies may be then used to screen cDNA expression libraries to isolate full-length cDNA clones of interest.

A genotype is the genetic constitution of an individual or group. Variations in genotype are essential in commercial breeding programs, in determining parentage, in diagnostics and fingerprinting, and the like. Genotypes can be readily described in terms of genetic markers. A genetic marker identifies a specific region or locus in the genome. The more genetic markers, the finer defined is the genotype. A genetic marker becomes particularly useful when it is allelic between organisms because it then may serve to unambiguously identify an individual. Furthermore, a genetic marker becomes particularly useful when it is based on nucleic acid sequence information that can unambiguously establish a genotype of an individual and when the function encoded by such nucleic acid is known and is associated with a specific trait. Such nucleic acids and/or nucleotide sequence information including single nucleotide polymorphisms (SNP's), variations in single nucleotides between allelic forms of such nucleotide sequence, can be used as perfect markers or candidate genes for the given trait.

Applicants have identified a number of SNP's of the nucleic acids or nucleic acid fragments of the present invention. These are indicated (marked with grey on the black background) in the figures that show multiple alignments of nucleotide sequences of nucleic acid fragments contributing to consensus contig sequences. See for example, Figures 3, 6, 11 and 14.

Accordingly, in a further aspect of the present invention, there is provided a substantially purified or isolated nucleic acid or nucleic acid fragment including a single nucleotide polymorphism (SNP) from a nucleic acid fragment shown in Figures 1 to 16 hereto, or complements or sequences antisense thereto.

In a still further aspect of the present invention there is provided a method of isolating a nucleic acid or nucleic acid fragment of the present invention including a single nucleotide polymorphism (SNP), said method including sequencing nucleic acid fragments from a nucleic acid library.

The nucleic acid library may be of any suitable type and is preferably a cDNA library.

The nucleic acid fragments may be isolated from recombinant plasmids or may be amplified, for example using polymerase chain reaction.

The sequencing may be performed by techniques known to those skilled in the art.

In a still further aspect of the present invention, there is provided use of nucleic acids or nucleic acid fragments of the present invention including SNP's, and/or nucleotide sequence information thereof, as molecular genetic markers.

In a still further aspect of the present invention there is provided use of a nucleic acid or nucleic acid fragment according to the present invention, and/or nucleotide sequence information thereof, as a molecular genetic marker.

More particularly, nucleic acids or nucleic acid fragments according to the present invention and/or nucleotide sequence information thereof may be used as a molecular genetic marker for quantitative trait loci (QTL) tagging, QTL mapping, DNA fingerprinting and in marker assisted selection, particularly in grasses and cereals. Even more particularly, nucleic acids or nucleic acid fragments according to the present invention and/or nucleotide sequence information thereof may be used as molecular genetic markers in forage and turf grass improvement, e.g. tagging QTLs for disease resistance, insect resistance, nematode resistance. Even more particularly, sequence information revealing SNPs in allelic variants of the nucleic acids or nucleic acid fragments of the present invention and/or nucleotide sequence information thereof may be used as molecular genetic markers for QTL tagging and mapping and in marker assisted selection, particularly in grasses and cereals.

In a still further aspect of the present invention there is provided a construct including a nucleic acid or nucleic acid fragment according to the present invention.

In a still further aspect of the present invention there is provided a vector including a nucleic acid or nucleic acid fragment according to the present

invention.

In a preferred embodiment of this aspect of the invention, the vector may include a regulatory element such as a promoter, a nucleic acid or nucleic acid fragment according to the present invention and a terminator; said regulatory element, nucleic acid or nucleic acid fragment and terminator being operatively linked.

The vector may be of any suitable type and may be viral or non-viral. The vector may be an expression vector. Such vectors include chromosomal, non-chromosomal and synthetic nucleic acid sequences, eg. derivatives of plant viruses; bacterial plasmids; derivatives of the Ti plasmid from *Agrobacterium tumefaciens*, derivatives of the Ri plasmid from *Agrobacterium rhizogenes*; phage DNA; yeast artificial chromosomes; bacterial artificial chromosomes; binary bacterial artificial chromosomes; vectors derived from combinations of plasmids and phage DNA. However, any other vector may be used as long as it is replicable, or integrative or viable in the plant cell.

The regulatory element and terminator may be of any suitable type and may be endogenous to the target plant cell or may be exogenous, provided that they are functional in the target plant cell.

Preferably the regulatory element is a promoter. A variety of promoters which may be employed in the constructs and vectors of the present invention are well known to those skilled in the art. Factors influencing the choice of promoter include the desired tissue specificity of the vector, and whether constitutive or inducible expression is desired and the nature of the plant cell to be transformed (eg. monocotyledon or dicotyledon). Particularly suitable promoters include but are not limited to the constitutive Cauliflower Mosaic Virus 35S (CaMV 35S) promoter and derivatives thereof, the maize Ubiquitin promoter, the rice Actin promoter, and the tissue-specific Arabidopsis small subunit (ASSU) promoter.

A variety of terminators which may be employed in the vectors and constructs of the present invention are also well known to those skilled in the art.

The terminator may be from the same gene as the promoter sequence or a different gene. Particularly suitable terminators are polyadenylation signals, such as the CaMV 35S polyA and other terminators from the nopaline synthase (*nos*), the octopine synthase (*ocs*) and the rbcS genes.

The vector, in addition to the regulatory element, the nucleic acid or nucleic acid fragment of the present invention and the terminator, may include further elements necessary for expression of the nucleic acid or nucleic acid fragment, in different combinations, for example vector backbone, origin of replication (ori), multiple cloning sites, recognition sites for recombination events, spacer sequences, enhancers, introns (such as the maize Ubiquitin *Ubi* intron), antibiotic resistance genes and other selectable marker genes [such as the neomycin phosphotransferase (*npt2*) gene, the hygromycin phosphotransferase (*hph*) gene, the phosphinothricin acetyltransferase (*bar* or *pat*) gene and the gentamycin acetyl transferase (*aaacC1*) gene], and reporter genes (such as beta-glucuronidase (GUS) gene (*gusA*) and green fluorescent proptein (gfp)]. The vector may also contain a ribosome binding site for translation initiation. The vector may also include appropriate sequences for amplifying expression.

As an alternative to use of a selectable marker gene to provide a phenotypic trait for selection of transformed host cells, the presence of the vector in transformed cells may be determined by other techniques well known in the art, such as PCR (polymerase chain reaction), Southern blot hybridisation analysis, histochemical GUS assays, visual examination including microscopic examination of fluorescence emitted by gfp, northern and Western blot hybridisation analyses.

Those skilled in the art will appreciate that the various components of the vector are operatively linked, so as to result in expression of said nucleic acid or nucleic acid fragment. Techniques for operatively linking the components of the vector of the present invention are well known to those skilled in the art. Such techniques include the use of linkers, such as synthetic linkers, for example including one or more restriction enzyme sites.

The constructs and vectors of the present invention may be incorporated

into a variety of plants, including monocotyledons (such as grasses from the genera *Deschampsia*, *Lolium*, *Festuca*, *Paspalum*, *Pennisetum*, *Panicum* and other forage and turfgrasses, corn, oat, sugarcane, wheat and barley), dicotyledons (such as arabidopsis, tobacco, white clover, red clover, subterranean clover, alfalfa, eucalyptus, potato, sugarbeet, canola, soybean, chickpea) and gymnosperms.

Techniques for incorporating the constructs and vectors of the present invention into plant cells (for example by transduction, transfection or transformation) are well known to those skilled in the art. Such techniques include *Agrobacterium*-mediated introduction, electroporation to tissues, cells and protoplasts, protoplast fusion, injection into reproductive organs, injection into immature embryos and high velocity projectile introduction to cells, tissues, calli, immature and mature embryos. The choice of technique will depend largely on the type of plant to be transformed.

Cells incorporating the constructs and vectors of the present invention may be selected, as described above, and then cultured in an appropriate medium to regenerate transformed plants, using techniques well known in the art. The culture conditions, such as temperature, pH and the like, will be apparent to the person skilled in the art. The resulting plants may be reproduced, either sexually or asexually, using methods well known in the art, to produce successive generations of transformed plants.

In a further aspect of the present invention there is provided a plant cell, plant, plant seed or other plant part, including, e.g. transformed with, a vector of the present invention.

The plant cell, plant, plant seed or other plant part may be from any suitable species, including monocotyledons, dicotyledons and gymnosperms.

The present invention also provides a plant, plant seed or other plant part, or a plant extract, derived from a plant cell of the present invention.

The present invention also provides a plant, plant seed or other plant part, or a plant extract, derived from a plant of the present invention.

In a further aspect of the present invention there is provided a method of modifying tolerance of freezing and/or low temperature stress in a plant, said method including introducing into said plant an effective amount of a nucleic acid or nucleic acid fragment, construct and/or a vector according to the present invention.

Using the methods and materials of the present invention, the tolerance of freezing and/or low temperature stress in a plant may be increased or decreased or otherwise modified. For example, the tolerance of freezing and/or low temperature stress may be increased or otherwise altered. They may be increased, for example, by incorporating additional copies of a sense nucleic acid or nucleic acid fragment of the present invention. They may be decreased, for example, by incorporating an antisense nucleic acid or nucleic acid fragment of the present invention.

The present invention will now be more fully described with reference to the accompanying Examples and drawings. It should be understood, however, that the description following is illustrative only and should not be taken in any way as a restriction on the generality of the invention described above.

In the Figures

Figure 1 shows the nucleotide sequence of DaIRIPa.

Figure 2 shows the deduced amino acid sequence of DaIRIPa.

Figure 3 shows the nucleotide sequences of the nucleic acid fragments contributing to the consensus contig sequence DaIRIPb.

Figure 4 shows the deduced amino acid sequence of DaIRIPb.

Figure 5 shows the consensus contig nucleotide sequence of DaIRIPb.

Figure 6 shows the nucleotide sequences of the nucleic acid fragments contributing to the consensus contig sequence DaIRIPc.

Figure 7 shows the deduced amino acid sequence of DaIRIPc.

Figure 8 shows the consensus nucleotide sequence of DaIRIPc.

Figure 9 shows the nucleotide sequence of DaIRIPd.

Figure 10 shows the deduced amino acid sequence of DaIRIPd.

Figure 11 shows the nucleotide sequences of the nucleic acid fragments contributing to the consensus contig sequence DaIRIPe.

Figure 12 shows the deduced amino acid sequence of DaIRIPe.

Figure 13 shows the consensus contig nucleotide sequence of DaIRIPe.

Figure 14 shows the nucleotide sequences of the nucleic acid fragments contributing to the consensus contig sequence DaIRIPf.

Figure 15 shows the deduced amino acid sequence of DaIRIPf.

Figure 16 shows the consensus contig nucleotide sequence of DaIRIPf.

EXAMPLE 1

Preparation of cDNA libraries, isolation and sequencing of cDNAs coding for IRIPs from from Antarctic hair-grass, *Deschampsia antarctica*.

cDNA librarires representing mRNAs from various organs and tissues from Antarctic hair-grass, *Deschampsia antarctica* were prepared. The characteristics of the libraries are described below (Table 1).

TABLE 1

cDNA libraries from Antarctic hair-grass, *Deschampsia antarctica.*

Library	Organ/Tissue
05Da	Aerial parts grown at 4°C
08Da	Roots grown at -15°C
09Da	Roots transferred from -15°C to 25°C for 24 h
10Da	Aerial parts transferred from -15°C to 25°C for 24 h
11Da	Aerial parts grown at -15°C
12Da	Roots grown at -15°C
15Da	Roots grown at 4°C
16Da	Aerial parts grown at 4°C
17Da	Roots transferred from 25°C to 0°C for 48 h
18Da	Aerial parts transferred from -15°C to 0°C for 48 h
19Da	Aerial parts transferred from 25°C to 0°C for 48 h, then to -15°C for 48 h

The cDNA libraries may be prepared by any of many methods available. For example, total RNA may be isolated using the Trizol method (Gibco-BRL, USA) or the RNeasy Plant Mini kit (Qiagen, Germany), following the manufacturers' instructions. cDNAs may be generated using the SMART PCR cDNA synthesis kit (Clontech, USA), cDNAs may be amplified by long distance polymerase chain reaction using the Advantage 2 PCR Enzyme system (Clontech, USA), cDNAs may be cleaned using the GeneClean spin column (Bio 101, USA), tailed and size fractionated, according to the protocol provided by Clontech. The cDNAs may be introduced into the pGEM-T Easy Vector system 1 (Promega, USA) according to the protocol provided by Promega. The cDNAs in the pGEM-T Easy plasmid vector are transfected into *Escherichia coli* Epicurian coli XL10-Gold ultra competent cells (Stratagene, USA) according to the protocol provided by Stratagene.

Alternatively, the cDNAs may be introduced into plasmid vectors for first preparing the cDNA libraries in Uni-ZAP XR vectors according to the manufacturer's protocol (Stratagene Cloning Systems, La Jolla, CA, USA). The

Uni-ZAP XR libraries are converted into plasmid libraries according to the protocol provided by Stratagene. Upon conversion, cDNA inserts will be contained in the plasmid vector pBluescript. In addition, the cDNAs may be introduced directly into precut pBluescript II SK(+) vectors (Stratagene) using T4 DNA ligase (New England Biolabs), followed by transfection into *E. coli* DH10B cells according to the manufacturer's protocol (GIBCO BRL Products).

Once the cDNA inserts are in plasmid vectors, plasmid DNAs are prepared from randomly picked bacterial colonies containing recombinant plasmids, or the insert cDNA sequences are amplified via polymerase chain reaction using primers specific for vector sequences flanking the inserted cDNA sequences. Plasmid DNA preparation may be performed robotically using the Qiagen QiaPrep Turbo kit (Qiagen, Germany) according to the protocol provided by Qiagen. Amplified insert DNAs are sequenced in dye-terminator sequencing reactions to generate partial cDNA sequences (expressed sequence tags or "ESTs"). The resulting ESTs are analyzed using an Applied Biosystems ABI 3700 sequence analyser.

EXAMPLE 2

DNA sequence analyses

The cDNA clones encoding IRIPs were identified by conducting BLAST (Basic Local Alignment Search Tool; Altschul *et al.* (1993) *J. Mol. Biol.* 215:403-410) searches. The cDNA sequences obtained were analysed for similarity to all publicly available DNA sequences contained in the eBioinformatics nucleotide database using the BLASTN algorithm provided by the National Center for Biotechnology Information (NCBI). The DNA sequences were translated in all reading frames and compared for similarity to all publicly available protein sequences contained in the SWISS-PROT protein sequence database using BLASTx algorithm (v 2.0.1) (Gish and States (1993) *Nature Genetics* 3:266-272) provided by the NCBI.

The cDNA sequences obtained and identified were then used to identify additional identical and/or overlapping cDNA sequences generated using the BLASTN algorithm. The identical and/or overlapping sequences were subjected to

a multiple alignment using the CLUSTALw algorithm, and to generate a consensus contig sequence derived from this multiple sequence alignment. The consensus contig sequence was then used as a query for a search against the SWISS-PROT protein sequence database using the BLASTx algorithm to confirm the initial identification.

Finally, it is to be understood that various alterations, modifications and/or additions may be made without departing from the spirit of the present invention as outlined herein.

It will also be understood that the term "comprises" (or its grammatical variants) as used in this specification is equivalent to the term "includes" and should not be taken as excluding the presence of other elements or features.

Documents cited in this specification are for reference purposes only and their inclusion is not acknowledgment that they form part of the common general knowledge in the relevant art.

Agriculture Victoria Services Pty Ltd
By their Registered Patent Attorneys
Freehills Carter Smith Beadle 24 November 2003

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                    *         20          *         40          *         60
DaIRIPa : GAGCTTCAACACTGTCGTAATTGGGAGTGACAATATCATAACCGGTAGCAAGCATGTCGT : 60

                    *         80          *        100          *        120
DaIRIPa : ATCTGGGAGGAAACATATCGTAACTGATAACAACAACAAAGTATCCGGGAATGACAATAA :120

                    *        140          *        160          *        180
DaIRIPa : TGTATCCGGGAGCTTCCACACCGTATCCGGGAGCCACAACACCGTATCCGGGAGCAACAA :180

                    *        200          *        220          *        240
DaIRIPa : TACCGTTTCCGGGAGCAACCATGTCGTGTCTGGGAGCAACAAAGTCGTGACAGGAGGTTA :240

                    *        260          *        280          *        300
DaIRIPa : ATTATGTGTCAGTGTAGGATTGTCTCCACCTGAGCTCACCCCTTGTCCAAATTGAGTCTA :300

                    *        320          *        340          *        360
DaIRIPa : GCTCACAATCAGTTGGTGGGGCCAATCGCGGCATGTAACTTCATGGATGGATATAGCATC :360

                    *        380          *        400          *
DaIRIPa : ATTTTCCCACTTTAAATAAAATTTGCCTCGTGGATGTCTAAAAAAAAAGA : 410
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FIGURE 1

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                      *         20         *         40         *         60
DaIRIPa : SFNTVVIGSDNIITGSKHVVSGRKHIVTDNNNKVSGNDNNVSGSFHTVSGSHNTVSGSNN : 60

                      *
DaIRIPa : TVSGSNHVVSGSNKVVTGG : 79
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FIGURE 2

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                   *        20         *        40         *        60
DaIRIPb1 : --------------------AACACAACACACTATTACGTTGGAGTGACGACAATGCCGT :  40
DaIRIPb2 : TCAGCAACGTTGNGACTGGATTTACATCACACTNCNACGTGGGAGTGACGACAATGCCGT :  60
DaIRIPb3 : ACAGCAACGGTGTGACTGGAAACACAACACACTATTACGTGGGAGTGACGACAATGCCGT :  60
DaIRIPb4 : ACAGCAACGTTGTGACTGGAACCACAACACACTATTACGTGGGAGTGACGACAATGCCGT :  60

                   *        80         *       100         *       120
DaIRIPb1 : AAGTGGTAGCAAGCATGTCGTATCTGGGACCCACCATGTCGTAACTGGCGACAACAATGC : 100
DaIRIPb2 : AAGTGGTTTCAAGCATGTCGTATNTGGGACCCACCATGTCGTAACTGGCGACAACAATGC : 120
DaIRIPb3 : AAGTGGTAGCAAGCATGTCGTATCTGGGACCCACCATGTCGTAACTGGCGACAACAATGC : 120
DaIRIPb4 : AAGTGGTAGCAAGCATGTCGTATCTGGGACCCACCATGTCGTAACTGGCGACAACAATGC : 120

                   *       140         *       160         *       180
DaIRIPb1 : CGTAACAAGGAACCACAATACCGTATCCGGGAGCCATAATACCGTACCTGGGAGCCATAA : 160
DaIRIPb2 : CGTAACAAGGAACCACAATACCGTATCCGGGAGCCATAATACCGTACCTGGGAGCCATAA : 180
DaIRIPb3 : CGTAACAAGGAACCACAATACCGTATCCGGGAGCCATAATACCGTACCTGGGAGCCATAA : 180
DaIRIPb4 : CGTAACAAGGAACCACAATACCGTATCCGGGAGCCATAATACCGTACCTGGGAGCCATAA : 180

                   *       200         *       220         *       240
DaIRIPb1 : TACCGTATCTGGGAGCCACAATACCGTATCTGGGAGCCACAATACCGTATCTGGAAGCAA : 220
DaIRIPb2 : TACCGTATCTGGGAGCCACAATACCGTATCTGGGAGCCACAATACCGTATCTGGAAGCNT : 240
DaIRIPb3 : TACCGTATCTGGGAGCCACAATACCGTATCTGGGAGCCACAATACCGTATCTGGAAGCAA : 240
DaIRIPb4 : TACCGTATCTGGGAGCCACAATACCGTATCTGGGAGCCACAATACCGTATCTGGAAGCAA : 240

                   *       260         *       280         *       300
DaIRIPb1 : CCACATCGTATCTGGGAACAACAAAGTCGTGACATGAGGTTAATGATCTTTAGTGGATTG : 280
DaIRIPb2 : TCACATCGTATCTGGGAACAACAAAGTCGTGACATGAGGTTAATGATCTTTAGTGGATTG : 300
DaIRIPb3 : CCACATCGTATCTGGGAACAACAAAGTCGTGACATGAGGTTAATGATCTTTAGTGGATTG : 300
DaIRIPb4 : CCACATCGTATCTGGGAACAACAAAGTCGTGACATGAGGTTAATGATCTTTAGTGGATTG : 300

                   *       320         *       340         *       360
DaIRIPb1 : TTTCCATCTTCCCTAACGAAGCTCATGTTCATGTCCAAGCTAATAAGTGTACCTCACAGT : 340
DaIRIPb2 : TTTCCATCTTCCCTAACGAAGCTCATGTTCATGTCCAAGCTAATAAGTGTACCTCACAGT : 360
DaIRIPb3 : TTTCCATCTTCCCTAACGAAGCTCATGTTCATGTCCAAGCTAATAAGTGTACCTCACAGT : 360
DaIRIPb4 : TTTCCATCTTCCCTAACGAAGCTCATGTTCATGTCCAAGCTAATAAGTGTACCTCACAGT : 360

                   *       380         *       400         *       420
DaIRIPb1 : CACTTGGTGGGGCCAATCGCGTTATGTAACTTGATGGATATAGCATCATTTTCGTACTTT : 400
DaIRIPb2 : CACTTGGTGGGGCCAATCGCGTTATGTAACTTGATGGATATAGCATCATTTTCGTACTTT : 420
DaIRIPb3 : CACTTGGTGGGGCCAATCGCGTTATGTAACTTGATGGATATAGCATCATTTTCGTACTTT : 420
DaIRIPb4 : CACTTGGTGGGGCCAATCGCGTTATGTAACTTGATGGATATAGCATCATTTTCGTACTTT : 420

                   *       440         *
DaIRIPb1 : AAATAAAACTCCCTTAAAAAACAAAAAAAAAA : 432
DaIRIPb2 : AAATAAAACTCCCTTAAAAAACAAAAAAAAAA : 452
DaIRIPb3 : AAATAAAACTCCCTTAAAAAACAAAAAAAAAA : 452
DaIRIPb4 : AAATAAAACTCCCTTAAAAAACAAAA------ : 446
```

FIGURE 3

```
                    *        20         *        40         *        60
DaIRIPb : QQRCDWKHNTLLRGSDDNAVSGSKHVVSGTHHVVTGDNNAVTRNHNTVSGSHNTVPGSHN : 60

                    *        80         *
DaIRIPb : TVSGSHNTVSGSHNTVSGSNHIVSGNNKVVT : 91
```

FIGURE 4

```
                     *        20         *        40         *        60
DaIRIPb : ACAGCAACGTTGTGACTGGAAACACAACACACTATTACGTGGGAGTGACGACAATGCCGT :  60

                     *        80         *       100         *       120
DaIRIPb : AAGTGGTAGCAAGCATGTCGTATCTGGACCCACCATGTCGTAACTGGCGACAACAATGC : 120

                     *       140         *       160         *       180
DaIRIPb : CGTAACAAGGAACCACAATACCGTATCCGGGAGCCATAATACCGTACCTGGGAGCCATAA : 180

                     *       200         *       220         *       240
DaIRIPb : TACCGTATCTGGGAGCCACAATACCGTATCTGGGAGCCACAATACCGTATCTGGAAGCAA : 240

                     *       260         *       280         *       300
DaIRIPb : CCACATCGTATCTGGGAACAACAAAGTCGTGACATGAGGTTAATGATCTTTAGTGGATTG : 300

                     *       320         *       340         *       360
DaIRIPb : TTTCCATCTTCCCTAACGAAGCTCATGTTCATGTCCAAGCTAATAAGTGTACCTCACAGT : 360

                     *       380         *       400         *       420
DaIRIPb : CACTTGGTGGGGCCAATCGCGTTATGTAACTTGATGGATATAGCATCATTTTCGTACTTT : 420

                     *       440         *
DaIRIPb : AAATAAAACTCCCTTAAAAAACAAAAAAAAAA : 452
```

FIGURE 5

```
                          *        20         *        40         *        60
DaIRIPc1 : AACAATGTTGTTTCCGGGAACGACAACACCGTCATATCTGGGAACAGGAACATTGTGTCT :  60
DaIRIPc2 : AACAATGTTGTTTCCGGG-ACGACAACACCGTCATATCTGGGAACAGGAACATTGTGTCT :  59

                          *        80         *       100         *       120
DaIRIPc1 : GGGAGCTACAACACCGTCGTAACTGGGAGTGATAATACCATAACCGGTAGCAACCATGTC : 120
DaIRIPc2 : GGGAGCTACAACACCGTCGTAACTGGGAGTGATAATACCATAACCGGTAGCAACCATGTC : 119

                          *       140         *       160         *       180
DaIRIPc1 : GTGTCTGGGAAGAACCATATCGTAACCGACAACAACAACGCCGTAACCGGGCACGACAAT : 180
DaIRIPc2 : GTGTCTGGGAAGAACCATATCGTAACCGACAACAACAACGCCGTAACCGGGCACGACAAT : 179

                          *       200         *       220         *       240
DaIRIPc1 : AATGTATCCGGGAGCTTCCATACCGTATCCGGGAACCACAACACAGTATCTGGGAGCAAT : 240
DaIRIPc2 : AATGTATCCGGGAGCTTCCATACCGTATCCGGGAACCACAACACAGTATCTGGGAGCAAT : 239

                          *       260         *       280         *       300
DaIRIPc1 : AATGCTGTATCAGGGAGCAACCATGTCGTGTCCGGGAGCAACAAAGTCGTGACAGGAGGT : 300
DaIRIPc2 : AATGCTGTATCAGGGAGCAACCATGTCGTGTCCGGGAGCAACAAAGTCGTGACAGGAGGT : 299

                          *       320         *       340         *       360
DaIRIPc1 : TAATCATATGTCCGTGCAGGATGCTTCCATGTTCCCTAAAGGAGATCGCGGCATTGTACA : 360
DaIRIPc2 : TAATGATATGTCCGTGCAGGATGCTTCCATGTTCCCTAAAGGAGATCGCGGCATTGTACA : 359

                          *       380         *       400         *       420
DaIRIPc1 : AGTTTTGTGTAGCTCACAATCACTTGGTGGGACCAATCGCGATGTCATGTAACTTCATGG : 420
DaIRIPc2 : AGTTTTGTGTAGCTCACAATCACTTGGTGGGACCAATCGCGATGTCATGTAACTTCATGG : 419

                          *       440         *       460         *
DaIRIPc1 : ATATAGCATCCTTTTCCTAATTTAAATAAAGTTTGCCTTGTGGAAAAAAAAAAA : 473
DaIRIPc2 : ATATAGCATCCTTTTCCTAATTTAAATAAAGTTTGNCTTGTGGA---------- : 463
```

FIGURE 6

```
                    *        20         *        40         *        60
DaIRIPc : NNVVSGNDNTVISGNRNIVSGSYNTVVTGSDNTITGSNHVVSGKNHIVTDNNNAVTGHDN : 60

                    *        80         *       100
DaIRIPc : NVSGSFHTVSGNHNTVSGSNNAVSGSNHVVSGSNKVVTGG : 100
```

FIGURE 7

```
              *        20         *        40         *        60
DaIRIPc : AACAATGTTGTTTCCGGGAACGACAACACCGTCATATCTGGGAACAGGAACATTGTGTCT :  60

              *        80         *       100         *       120
DaIRIPc : GGGAGCTACAACACCGTCGTAACTGGGAGTGATAATACCATAACCGGTAGCAACCATGTC : 120

              *       140         *       160         *       180
DaIRIPc : GTGTCTGGGAAGAACCATATCGTAACCGACAACAACAACGCCGTAACCGGGCACGACAAT : 180

              *       200         *       220         *       240
DaIRIPc : AATGTATCCGGGAGCTTCCATACCGTATCCGGGAACCACAACACAGTATCTGGGAGCAAT : 240

              *       260         *       280         *       300
DaIRIPc : AATGCTGTATCAGGGAGCAACCATGTCGTGTCCGGGAGCAACAAAGTCGTGACAGGAGGT : 300

              *       320         *       340         *       360
DaIRIPc : TAATGATATGTCCGTGCAGGATGCTTCCATGTTCCCTAAAGGAGATCGCGGCATTGTACA : 360

              *       380         *       400         *       420
DaIRIPc : AGTTTTGTGTAGCTCACAATCACTTGGTGGGACCAATCGCGATGTCATGTAACTTCATGG : 420

              *       440         *       460         *
DaIRIPc : ATATAGCATCCTTTTCCTAATTTAAATAAAGTTTGCCTTGTGGAAAAAAAAAA : 473
```

FIGURE 8

```
                 *        20         *        40         *        60
DaIRIPd : GACAACACTTGCGAATCACTTGCATTCCAAAAAAGTCCATTCCTGAGTTGCATACCACAG :  60

                 *        80         *       100         *       120
DaIRIPd : CTGAATCCATGGCGCCGCGTGGTCCGGCGCCTCATGCTGCGACTGGGAAGGCGTGAGCAT : 120

                 *       140         *       160         *       180
DaIRIPd : CCTTGGCGGGCCTCACGCGGCATGTGAAAGGTAACAGGAGAACACTTGCCGTACAACCGA : 180

                 *       200         *       220         *       240
DaIRIPd : ATACAATTACTGGGACCAACAACAACGTCAGGTCTGGGAGCAACAATGTTGTTTCCGGGA : 240

                 *       260         *       280         *       300
DaIRIPd : ACGACAACACCGTCATATCTGGGAACAGGAACATTGTGTCTGGGAGCTACAACACCGTCG : 300

                 *       320         *       340         *       360
DaIRIPd : TAACTGGGAGTGATAATACCATAACCGGTAGCAACCATGTCGTGTCTGGGAAGAACCATA : 360

                 *       380         *       400         *       420
DaIRIPd : TCGTAACCGACAACAACAACGCCGTAACCGGGCACGACAATAATGTATCCGGGAGCTTCC : 420

                 *       440         *       460         *       480
DaIRIPd : ATACCGTATCCGGGAACCACAACACAGTATCTGGGAGCAATAATACTGTATCAGGGAGCA : 480

                 *       500         *       520         *       540
DaIRIPd : ACCATGTCGTGTCCGGGAGCAACAAAGTCGTGACAGGAGGTTAATGATATGTCCGTGCAG : 540

                 *       560         *       580         *       600
DaIRIPd : GATGCTTCCATGTTCCCTAAAGGAGATCGCGGCATTGTACAAGTTTTGTGTAGCTCACAA : 600

                 *       620         *       640         *       660
DaIRIPd : TCACTTGGTGGGACCAATCGCGATGTCATGTAACTTCATGGATATAGCATCCTTTTCCTA : 660

                 *       680         *
DaIRIPd : ATTTAAATAAAGTTTGCCTTGTGTAAAAAAAAAAA : 695
```

FIGURE 9

```
                 *        20         *        40         *        60
DaIRIPd : ASLAGLTRHVKGNRRTLAVQPNTITGTNNNVRSGSNNVVSGNDNTVISGNRNIVSGSYNT : 60

                 *        80         *       100         *       120
DaIRIPd : VVTGSDNTITGSNHVVSGKNHIVTDNNNAVTGHDNNVSGSFHTVSGNHNTVSGSNNTVSG :120

                 *
DaIRIPd : SNHVVSGSNKVVTGG : 135
```

FIGURE 10

```
                        *        20         *        40         *        60
DaIRIPe1 : CGATTAAGCAGTGGTAACAACGCAGAGTACGCGGGGAG-CCAAGGAACACTTACGAATCAC :  60
DaIRIPe2 : -------------------------------------GACCAAGGAACACTTACGAATCAC :  24
DaIRIPe3 : -------------------------------------GACC-AGGAACACTTACGAATCAC :  23

                  *        80         *       100         *       120
DaIRIPe1 : TTGCATTCCAAAGAAGGTTTCTTACTCAGTTGTTGCGTCTGTGTATGCATAGCGTAACACA : 121
DaIRIPe2 : TTGCATTCCAAAGAAGGTTTCTTACTCAGTTGTTGCGTCTGTGTATGCATAGCGTAACACA :  85
DaIRIPe3 : TTGCATTCCAAAGAAGGTTTCTTACTCAGTTGTTGCGTCTGTGTATGCATAGCGTAACACA :  84

                *       140         *       160         *       180
DaIRIPe1 : GCTTGAGTCCATGGCGAACTGCTGTCTGCTACTCCTCTTCTTGGCGCTACTCTTGCCTGCG : 182
DaIRIPe2 : GCTTGAGTCCATGGCGAACTGCTGTCTGCTACTCCTCTTCTTGGCGCTACTCTTGCCTGCG : 146
DaIRIPe3 : GCTTGAGTCCATGGCGAACTGCTGTCTGCTACTCCTCTTCTTGGCGCTACTCTTGCCTGCG : 145

               *       200         *       220         *       240
DaIRIPe1 : GCTGGGAAGGCGTGGGCTGCGACAGCGCAAGCGGCCGCGTCACGGCGATGTTGCTCCCCAG : 243
DaIRIPe2 : GCTGGGAAGGCGTGGGCTGCGACAGCGCAAGCGGCCGCGTCACGGCGATGTTGCTCCCCAG : 207
DaIRIPe3 : GCTGGGAAGGCGTGGGCTGCGACAGCGCAAGCGGCCGCGTCACGGCGATGTTGCTCCCCAG : 206

              *       260         *       280         *       300
DaIRIPe1 : GCACGGCCTCGCGAAGCCCGTCCCAGGAGCATCCTTGGCGAGCCTCGCACGGCTAGAGGAG : 304
DaIRIPe2 : GCACGGCCTCGCGAAGCCCGTCCCAGGAGCATCCTTGGCGAGCCTCGCACGGCTAGAGGAG : 268
DaIRIPe3 : GCACGGCCTCGCGAAGCCCGTCCCAGGAGCATCCTTGGCGAGCCTCGCACGGCTAGAGGAG : 267

             *       320         *       340         *       360
DaIRIPe1 : CTCTTCAAGCGTAACAGAAGAACACTGGAGGAACAGCCAAATACAATTCAAGGGACCAACA : 365
DaIRIPe2 : CTCTTCAAGCGTAACAGAAGAACACTGGAGGAACAGCCAAATACAATTCAAGGGACCAACA : 329
DaIRIPe3 : CTCTTCAAGCGTAACAGAAGAACACTGGAGGAACAGCCAAATACAATTCAAGGGACCAACA : 328

            *       380         *       400         *       420
DaIRIPe1 : ACAATGTCAGAGATGGGTGCTACAATGCTCTTTCTGGAAATGACAACACTGTCATATCCGG : 426
DaIRIPe2 : ACAATGTCAGAGATGGGTGCTACAATGCTCTTTCTGGAAATGACAACACTGTCATATCCGG : 390
DaIRIPe3 : ACAATGTCAGAGATGGGTGCTACAATGCTCTTTCTGGAAATGACAACACTGTCATATCCGG : 389

           *       440         *       460         *       480
DaIRIPe1 : AAACAACAACACTGTGTCTGGGAGCTTTAACACTATCGTAACTGGGTGTCACAACACTGTG : 487
DaIRIPe2 : AAACAACAACACTGTGTCTGGGAGCTTTAACACTATCGTAACTGGGTGTCACAACACTGTG : 451
DaIRIPe3 : AAACAACAACACTGTGTCTGGGAGCTTTAACACTATCGTAACTGGGTGTCACAACACTGTG : 450

          *       500         *       520         *       540
DaIRIPe1 : TCTGGTAGCAACCAGGTTGTGTCCGGGCTCAACCATATCGTAACTGACGACAACAATGACG : 548
DaIRIPe2 : TCTGGTAGCAACCAGGTTGTGTCCGGGCTCAACCATATCGTAACTGACGACAACAATGACG : 512
DaIRIPe3 : TCTGGTANCAACCAGGTTGTGTCCGGGCTCAACCATATCGTAACTGACGACAACAATGACG : 511
```

FIGURE 11

```
              *       560         *       580         *       600         *
DaIRIPe1 : TATCAGGTAACGATAATAATGTATCCGGTAGCTTTCATACCGTATCTGGGAGCCACAATAC : 609
DaIRIPe2 : TATCAGGTAACGATAATAATGTATCCGGTAGCTTTCATACCGTATCTGGGAGCCACAATAC : 573
DaIRIPe3 : TATCAGGTAACGATAATAATGTATCCGGTAGCTTTCATACCGTATCTGGGAGCCACAATAC : 572

                  620         *       640         *       660         *
DaIRIPe1 : CGTATCTGGGAGCAACAATACCGTATCTGGGAGAAACCATGTCGTAACTGGGAGTAACAAA : 670
DaIRIPe2 : CGTATCTGGGAGCAACAATACCGTATCTGGGAGAAACCATGTCGTAACTGGGAGTAACAAA : 634
DaIRIPe3 : CGTATCTGGGAGCAACAATACCGTATCTGGGAGAAACC----------------------- : 610

                680         *       700         *       720         *
DaIRIPe1 : GTCGTGACAGGAGGTTAATGATCAGTGAGTGGATTGTTTCCATCTTCACTAACGAAGCTTA : 731
DaIRIPe2 : ------------------------------------------------------------- :   -
DaIRIPe3 : ------------------------------------------------------------- :   -

              740         *       760         *       780         *
DaIRIPe1 : CGCCCTTGTCCAAGTTCAACCTAGAGCTCACAATATCTTGGTGGGCCCAATCGTCTTATGT : 792
DaIRIPe2 : ------------------------------------------------------------- :   -
DaIRIPe3 : ------------------------------------------------------------- :   -

            800         *       820         *       840         *
DaIRIPe1 : AACTTCATGGATGTATCCTCCTTTTCCTACTTTAAATAAATTTCCTTAAAATGTCTTCCAA : 853
DaIRIPe2 : ------------------------------------------------------------- :   -
DaIRIPe3 : ------------------------------------------------------------- :   -

          860
DaIRIPe1 : AAAAAAAAA : 862
DaIRIPe2 : --------- :   -
DaIRIPe3 : --------- :   -
```

FIGURE 11 (cont..)

```
                    *        20         *        40         *        60
DaIRIPe : MLLPRHGLAKPVPGASLASLARLEELFKRNRRTLEEQPNTIQGTNNNVRDGCYNALSGND : 60

                    *        80         *       100         *       120
DaIRIPe : NTVISGNNNTVSGSFNTIVTGCHNTVSGSNQVVSGLNHIVTDDNNDVSGNDNNVSGSFHT :120

                    *       140         *
DaIRIPe : VSGSHNTVSGSNNTVSGRNHVVTGSNKVVTGG : 152
```

FIGURE 12

```
                    *        20         *        40         *        60
DaIRIPe : CGATTAAGCAGTGGTAACAACGCAGAGTACGCGGGGAGACCAAGGAACACTTACGAATCA :  60

                    *        80         *       100         *       120
DaIRIPe : CTTGCATTCCAAAGAAGGTTTCTTACTCAGTTGTTGCGTCTGTGTATGCATAGCGTAACA : 120

                    *       140         *       160         *       180
DaIRIPe : CAGCTTGAGTCCATGGCGAACTGCTGTCTGCTACTCCTCTTCTTGGCGCTACTCTTGCCT : 180

                    *       200         *       220         *       240
DaIRIPe : GCGGCTGGGAAGGCGTGGGCTGCGACAGCGCAAGCGGCCGCGTCACGGCGATGTTGCTCC : 240

                    *       260         *       280         *       300
DaIRIPe : CCAGGCACGGCCTCGCGAAGCCCGTCCCAGGAGCATCCTTGGCGAGCCTCGCACGGCTAG : 300

                    *       320         *       340         *       360
DaIRIPe : AGGAGCTCTTCAAGCGTAACAGAAGAACACTGGAGGAACAGCCAAATACAATTCAAGGGA : 360

                    *       380         *       400         *       420
DaIRIPe : CCAACAACAATGTCAGAGATGGGTGCTACAATGCTCTTTCTGGAAATGACAACACTGTCA : 420

                    *       440         *       460         *       480
DaIRIPe : TATCCGGAAACAACAACACTGTGTCTGGGAGCTTTAACACTATCGTAACTGGGTGTCACA : 480

                    *       500         *       520         *       540
DaIRIPe : ACACTGTGTCTGGTAGCAACCAGGTTGTGTCCGGGCTCAACCATATCGTAACTGACGACA : 540

                    *       560         *       580         *       600
DaIRIPe : ACAATGACGTATCAGGTAACGATAATAATGTATCCGGTAGCTTTCATACCGTATCTGGGA : 600

                    *       620         *       640         *       660
DaIRIPe : GCCACAATACCGTATCTGGGAGCAACAATACCGTATCTGGGAGAAACCATGTCGTAACTG : 660

                    *       680         *       700         *       720
DaIRIPe : GGAGTAACAAAGTCGTGACAGGAGGTTAATGATCAGTGAGTGGATTGTTTCCATCTTCAC : 720

                    *       740         *       760         *       780
DaIRIPe : TAACGAAGCTTACGCCCTTGTCCAAGTTCAACCTAGAGCTCACAATATCTTGGTGGGGCC : 780

                    *       800         *       820         *       840
DaIRIPe : AATCGTCTTATGTAACTTCATGGATGTATCCTCCTTTTCCTACTTTAAATAAATTTCCTT : 840

                    *       860
DaIRIPe : AAAATGTCTTCCAAAAAAAAAAA : 863
```

FIGURE 13

```
                   *        20         *        40         *        60
DaIRIPf1 : CCCCAGGCGCGGCCTCGCGGGCCCCATCACAGGAGCAACCTTGGCCGGCCTGACACGGCT :  60
DaIRIPf2 : GCCCAGGCGCGG-CTCGCGG-CCCATCACAGGAGC-A-CTTGGCCGGCCTGACACGGCT :  56
DaIRIPf3 : CCCCAGGCGCGGCCTCGCGGGCCCCATCACAGGAGCAACCTTGGCCGGCCTGACACGGCT :  60

                   *        80         *       100         *       120
DaIRIPf1 : TGAGTCGCTCAACCTTGCCANCAACAGTCTGGTAGGCACCATCCCATCATGGATCGGTGA : 120
DaIRIPf2 : TGAGTCGCTCAACCTTGCCAACAACAGTCTGGTAGGCACCATCCCATCATGGATCGGTGA : 116
DaIRIPf3 : TGAGTCGCTCAACCTTGCCAACAACAGTCTGGTAGGCACCATCCCATCATGGATCGGTGA : 120

                   *       140         *       160         *       180
DaIRIPf1 : GCTTGACCACCTTTGCTACATGGATCTCTCACACAATTCACTAGATGGCGAGGTACCCAA : 180
DaIRIPf2 : GCTTGACCACCTTTGCTACATGGATCTCTCACACAATTCACTAGATGGCGAGGTACCCAA : 176
DaIRIPf3 : GCTTGACCACCTTTGCTACATGGATCTCTCACACAATTCACTAGATGGCGAGGTACCCAA : 180

                   *       200         *       220         *       240
DaIRIPf1 : GAGTTTGCAGATACGGCTCAGGGCCCTCACTACGACCGGTCGTTCACTGGGCATGGTTTT : 240
DaIRIPf2 : GAGTTTGCAGATACGGCTCAGGGCCCTCACTACGACCGGTCGTTCACTGGGCATGGTTTT : 236
DaIRIPf3 : GAGTTTGCAGATACGGCTCAGGGCCCTCACTACGACCGGTCGTTCACTGGGCATGGTTTT : 240

                   *       260         *       280         *       300
DaIRIPf1 : CATTAACATGCCGTTGCATATGAAGCGTAGCCGAAGAACACTCCAAGAACAACCAAATGT : 300
DaIRIPf2 : CATTAACATGCCGTTGCATATGAAGCGTAGCCGAAGAACACTCCAAGAACAACCAAATGT : 296
DaIRIPf3 : CATTAACATGCCGTTGCATATGAAGCGTAGCCGAAGAACACTCCAAGAACAACCAAATGT : 300

                   *       320         *       340         *       360
DaIRIPf1 : AATAACTGGACCAACAACAGTGTCAGATCTGGGAGAAACAATGTTGTTTCCGGGAACGA : 360
DaIRIPf2 : AATAACTGGACCAACAACAGTGTCAGATCTGGGAGAAACAATGTTGTTTCCGGGAACGA : 356
DaIRIPf3 : AATAACTGGACCAACAACAGTGTCAGATCTGGGAGAAACAATGTTGTTTCCGGGAACGA : 360

                   *       380         *       400         *       420
DaIRIPf1 : CAATACTGTCATATCTGGGAACAACAATGTTGTGTCTGGGAGCCACAACACTGTCGTAAC : 420
DaIRIPf2 : CAATACTGTCATATCTGGCAACAACAATGTTGTGTCTGGGAGCCACAACACTGTCGTAAC : 416
DaIRIPf3 : CAATACTGTCATATCTGCGAACAACAATGTTGTGTCTGGGAGCCACAACACTGTCGTAAC : 420

                   *       440         *       460         *       480
DaIRIPf1 : GGGGAGTGACAATGTCGTAAGTGGTAGTAACCATGTCGTATCTAGGACCAACCATGTCGT : 480
DaIRIPf2 : GGGGAGTGACAATGTCGTAAGTGGTAGTAACCATGTCGTATCTAGGACCAACCATGTCGT : 476
DaIRIPf3 : GGGGAGTGACAATGTCGTAAGTGGTAGTAACCATGTCGTATCTAGGACCAACCATGTCGT : 480

                   *       500         *       520         *       540
DaIRIPf1 : AACTGATAACAACAATGCCGTAACCGGGAACCACAACACTGTATCCGGGAGCCACAACAC : 540
DaIRIPf2 : AACTGATAACAACAATGCCGTAACCGGGAACCACAACACTGTATCCGGGAGCCACAACAC : 536
DaIRIPf3 : AACTGATAACAACAATGCCGTAACCGGGAACCACAACACTGTATCCGGGAGCCACAACAC : 540

                   *       560         *       580         *       600
DaIRIPf1 : TGTATCCGGGAGCAACAATGTCGTATCCGGGAGCAACCATGTTGTATCAGGGAGCAACAA : 600
DaIRIPf2 : TGTATCCGGGAGCAACAATGTCGTATCCGGGAGCAACCATGTTGTATCAGGGAGCAACAA : 596
DaIRIPf3 : TGTATCCGGGAGCAACAATGTCGTATCCGGGAGCAACCATGTTGTATCAGGGAGCAACAA : 600
```

FIGURE 14

```
                      *        620         *        640         *        660
DaIRIPf1 : AGTCGTGACGGGAGGTTAATTAATGATC-------------------------------- : 628
DaIRIPf2 : AGTCGTGACGGGAGGTTAATTAATGATCTATCAGTGGATTGTCTCCATCGTCCCTGACGG : 656
DaIRIPf3 : AGTCGTGACGGGAGGTTAATTAATGATCTATCAGTGCATTGTCTCCATCGTCCCTGACGG : 660

                      *        680         *        700         *        720
DaIRIPf1 : ------------------------------------------------------------ :   -
DaIRIPf2 : AGTTCACGTCCTTGTCCAAGTTCAGTGTAGCTTACAATCACATGGTAGGGCCAATCGCAT : 716
DaIRIPf3 : AGTTCACGTCCTTGTCCAAGTTCAGTGTAGCTTACAATCACATGGTAGGGCCAATCGCAT : 720

                      *        740         *        760         *        780
DaIRIPf1 : ------------------------------------------------------------ :   -
DaIRIPf2 : TATGTAACTTCATGGATATAGCATCC---------------------------------- : 742
DaIRIPf3 : TATGTAACTTCATGGATATAGCATCCTTTTTCTGTTTTAAATAAAAACCCCTAAACTATC : 780

                      *
DaIRIPf1 : --------------- :   -
DaIRIPf2 : --------------- :   -
DaIRIPf3 : TTACAAAAAAAAAAA : 795
```

FIGURE 14 (cont..)

```
                    *         20         *         40         *         60
DaIRIPf : MDLSHNSLDGEVPKSLQIRLRALTTTGRSLGMVFINMPLHMKRSRRTLQEQPNVITGTNN :  60

                    *         80         *        100         *        120
DaIRIPf : SVRSGRNNVVSGNDNTVISGNNNVVSGSHNTVVTGSDNVVSGSNHVVSRTNHVVTDNNNA : 120

                    *        140         *
DaIRIPf : VTGNHNTVSGSHNTVSGSNNVVSGSNHVVSGSNKVVTGG : 159
```

FIGURE 15

```
                 *        20         *        40         *        60
DaIRIPf : CCCCAGGCGCGGCCTCGCGGGCCCCATCACAGGAGCAACCTTGGCCGGCCTGACACGGCT :  60

                 *        80         *       100         *       120
DaIRIPf : TGAGTCGCTCAACCTTGCCAACAACAGTCTGGTAGGCACCATCCCATCATGGATCGGTGA : 120

                 *       140         *       160         *       180
DaIRIPf : GCTTGACCACCTTTGCTACATGGATCTCTCACACAATTCACTAGATGGCGAGGTACCCAA : 180

                 *       200         *       220         *       240
DaIRIPf : GAGTTTGCAGATACGGCTCAGGGCCCTCACTACGACCGGTCGTTCACTGGGCATGGTTTT : 240

                 *       260         *       280         *       300
DaIRIPf : CATTAACATGCCGTTGCATATGAAGCGTAGCCGAAGAACACTCCAAGAACAACCAAATGT : 300

                 *       320         *       340         *       360
DaIRIPf : AATAACTGGGACCAACAACAGTGTCAGATCTGGGAGAAACAATGTTGTTTCCGGGAACGA : 360

                 *       380         *       400         *       420
DaIRIPf : CAATACTGTCATATCTGGGAACAACAATGTTGTGTCTGGGAGCCACAACACTGTCGTAAC : 420

                 *       440         *       460         *       480
DaIRIPf : GGGGAGTGACAATGTCGTAAGTGGTAGTAACCATGTCGTATCTAGGACCAACCATGTCGT : 480

                 *       500         *       520         *       540
DaIRIPf : AACTGATAACAACAATGCCGTAACCGGGAACCACAACACTGTATCCGGGAGCCACAACAC : 540

                 *       560         *       580         *       600
DaIRIPf : TGTATCCGGGAGCAACAATGTCGTATCCGGGAGCAACCATGTTGTATCAGGGAGCAACAA : 600

                 *       620         *       640         *       660
DaIRIPf : AGTCGTGACGGGAGGTTAATTAATGATCTATCAGTGGATTGTCTCCATCGTCCCTGACGG : 660

                 *       680         *       700         *       720
DaIRIPf : AGTTCACGTCCTTGTCCAAGTTCAGTGTAGCTTACAATCACATGGTAGGGCCAATCGCAT : 720

                 *       740         *       760         *       780
DaIRIPf : TATGTAACTTCATGGATATAGCATCCTTTTTCTGTTTAAATAAAAACCCCTAAACTATC : 780

                 *
DaIRIPf : TTACAAAAAAAAAAA : 795
```

FIGURE 16